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For the President of the European Patent Office

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Anmelder/Applicant(s)/Demandeur(s):

NESTEC S.A.
Avenue Nestlé 55
1800 Vevey
SUISSE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:

(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.

If no title is shown please refer to the description.

Si aucun titre n'est indiqué se référer à la description.)

Nutritional composition for wound healing

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FINAL DRAFT

**Patent Application
In the name of Nestec S.A.**

Title: Nutritional Composition for Wound Healing

Inventors: Hans Smola

Enteral Composition for Wound Healing**Field of the invention**

- 5 This invention relates to a nutritional composition for promoting wound healing, particularly the healing of chronic wounds such as pressure ulcers (decubitus).

Background of the invention

- 10 In normal wound healing, there are three phases which overlap to some extent. Briefly, the first phase is inflammation in which the clot forms and stops the bleeding from blood vessels followed by extravasation of mononuclear blood cells which clean the wound and remove debris. The next phase is the granulation phase in which fibroblasts proliferate and accumulate in the wound and produce collagen to assist in
- 15 wound closure. This phase is characterised by high metabolic activity. Finally, epithelial cells begin to cover the wound.

- Delayed or impaired wound healing is a problem for health care professionals and patients as it results in increased treatment times and stays in healthcare facilities and
- 20 distress to patients. The process of wound healing can be interrupted in any of the phases described above as a result of factors such as infection or malnutrition. The pressure ulcers which frequently afflict elderly and bed-ridden patients are a particular concern and these categories of patients are often found to be suffering from malnutrition. Indeed, all patients with acute or chronic wounds exhibit
- 25 increased nutritional requirements, displaying a need for increased nutrients and energy as compared with individuals not challenged by such metabolic stresses. If these patients are malnourished before suffering wounds, the wounds may simply fail to heal.

- 30 In recent years, much attention has focused on the role of arginine in wound healing. This is discussed for example in USP 5,053,387 which discloses an enteral nutritional formulation in which 1 to 3% of the total energy intake is preferably provided by arginine. Similarly, EP 960 572 A discloses a nutritional composition suitable for the treatment and prevention of pressure ulcers which includes arginine as well as large
- 35 amounts of vitamins C and E. The role of arginine is also discussed in USP 5,733,884 which discloses a method of providing nutrition to a patient with an acute or chronic wound using a composition in which at least 2% of the energy is provided

by arginine and the same amount by proline. This patent hypothesises that arginine and proline have a synergistic effect in enhancing wound healing. Commercially, there are a number of products marketed as suitable for promoting wound healing on the basis that they contain high levels of arginine including CUBITAN® and ARGINAID®.

An adequate supply of arginine is clearly relevant to the wound healing process. However, arginine is also a precursor for the formation of nitric oxide which acts as a vasodilator and enhances growth hormone secretion. It is not desirable for critically ill individuals to be exposed to high amounts of nitric oxide and yet this will inevitably happen if such individuals receive nutritional supplements containing high levels of arginine – see, for example L. Cynober, Curr Opin Clin Nutr Metab Care. 6:189-93 2003. Moreover, it is quite likely that a high proportion of elderly, bedridden or critically ill patients at risk of developing pressure sores will also suffer from conditions for which high levels of nitric oxide are contra-indicated (J. Takala et al., N Engl J Med 341:785-792 1999).

Summary of the Invention

In a first aspect, the present invention provides a nutritional composition for promoting wound healing comprising a protein source, a lipid source and a carbohydrate source wherein no more than 1.8% of the total calories of the composition derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the composition.

In a second aspect, the present invention provides a method of providing nutritional support to a patient with an acute or chronic wound comprising the step of administering a therapeutically effective amount of a nutritional composition comprising a protein source, a lipid source and a carbohydrate source wherein no more than 1.8% of the total calories of the composition derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the composition.

In a third aspect, the present invention provides the use of a protein source, a lipid source and a carbohydrate source for the manufacture of a therapeutic formulation for promoting wound healing wherein no more than 1.8% of the total calories of the

formulation derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the formulation.

Detailed Description of the Invention

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Although the inflammatory phase of the wound healing process described above is critical, the present inventor believes that from a therapeutic/nutritional approach, attempts to modulate this phase carry high risks and that the granulation phase offers better potential for nutritional intervention. In this phase, new connective tissue is synthesised and more than 80% of this tissue is composed of collagen. Collagen is rich in the amino acids proline (about 22%) and glycine (about 33%) and the presence of these amino acids is rate limiting for collagen formation, that is to say, collagen cannot be efficiently formed if they are not available in sufficient quantity. However, the normal diet contains only about 3% in total of these amino acids and it will be appreciated that individuals who have suffered wounds may ingest even less of them, particularly as proline is not generally regarded as an essential dietary amino acid. In the case of individuals suffering from malnutrition for whatever reason, these shortages may be particularly pronounced. The composition of the present invention is therefore supplemented with proline in a quantity sufficient to facilitate collagen synthesis. It is particularly suitable for the amelioration of pressure ulcers but may also be used in the management of acute wounds including before and after surgery.

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The composition of the present invention does not need to be supplemented with arginine - of course some arginine is likely to be present as part of the protein source. However, it is widely believed that arginine also has a role in the inflammatory phase of wound healing and, for this reason, the composition of the present invention is preferably supplemented with small amounts of arginine subject always to the requirement that arginine must account for no more than 1.8% of the total calories of the composition..

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The composition of the present invention contains sources of protein, lipids and carbohydrate and may be administered orally or enterally. The composition preferably provides about 1.25 kcal/ml.

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Protein is essential to healing as tissue damage results in a catabolic response that includes a requirement for a larger proportion of total calories as protein than is

required by the general population. Research suggests that enteral fortification employing large quantities of protein can accelerate the synthesis of visceral proteins and so the protein source of the present invention preferably constitutes at least 25% of the total energy content of the composition, more preferably at least 28%.

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A variety of different protein sources may be used including intact protein sources such as casein or whey as well as hydrolysed proteins, free amino acids and even mixtures of intact and hydrolysed proteins and/or free amino acids, in each case supplemented with free proline and, optionally, free arginine. Preferably, the protein source of the present invention is selected to yield the highest amount of proline in the proteins so as to minimise the amount that needs to be added as the free amino acid.

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Preferably, proline constitutes at least 3.5% of the calories of the composition of the present invention. At this level of contribution to total calories, the composition will need to be supplemented by about 3.0% (by weight of the protein source) proline.

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The total calories/gram of nitrogen of the composition of the invention is preferably about 160:1. The total non-protein calories/ gram of nitrogen is preferably about 110:1.

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The composition of the present invention also includes a lipid source. Lipids or fats are the primary source of stored energy in the body and energy from fat metabolism is used in all normal cell functions. As far as wound healing is concerned, fat metabolism results in the formation of prostaglandins and other regulators of the inflammatory process. The lipid source used in the present invention preferably constitutes about 20% of the total energy content of the composition. Of this 20%, preferably about 8% is constituted by mono- and di-glycerides of fatty acids. The ratio of n-6 to n-3 fatty acids is preferably between 4:1 and 10:1, more preferably about 7:1.

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The composition of the present invention also includes a carbohydrate source. Glucose is the primary fuel for cellular metabolism of many tissues including leucocytes, macrophages and fibroblasts all of which are involved in the wound healing process. Glucose is needed to meet the specific metabolic demands of wound healing. The carbohydrate source used in the present invention preferably constitutes

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about 50% of the total energy content of the composition. Suitable sources of carbohydrate are maltodextrin and sucrose. Preferably, the carbohydrate source is substantially free of lactose.

5 Vitamins, minerals and trace elements are also important in the wound healing process. Preferably, the composition of the present invention at least complies with the compositional criteria set out in Directive 1999/21/EC on Dietary Foods for Special Medical Purposes as regards these micronutrients. However, certain micronutrients are particularly important for wound healing and therefore the
10 composition of the present invention preferably contains more than the recommended minimum levels of vitamins C and E, manganese, zinc and selenium.

A liquid, ready to use composition according to the present invention will now be given by way of example:-

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Example 1

	Caloric density	1.25g/ml	
	Protein	30% of kcal	
20	of which (by weight):-	sodium caseinate	50%
		milk protein concentrate	45%
		free L-proline	3%
		free L-arginine	2%
	total L-proline	12.4% of protein source	
25	total L-arginine	5.0% of protein source	
	Caloric contribution of total proline	3.7%	
	Caloric contribution of total arginine	1.5%	
	Lipids	20% of kcal	
30	of which	rapeseed oil	35%
		corn oil	34%
		soya oil	20%
		mono and di-glycerides of fatty acids	8%
		milk fat	3%
35	n-6:n-3	7.2:1	

	Carbohydrate	50% of kcal	
	of which	corn syrup	52%
		sucrose	43%
5		starch	3%
		lactose	2%
	Vitamin C	125mg/100ml	
	Vitamin E	7.5mg α -tocopherol equivalents/100ml	
	Manganese	1.9mg/100ml	
	Zinc	3.7mg/100ml	
10	Selenium	19 μ g/100ml	
	Osmolarity	470 mosm/Kg water	
	Water	80.3%	
	Density	1.087g/ml	
15	Total cal/g nitrogen	160:1	
	Non-protein cal/g nitrogen	110:1	

As will be appreciated from the foregoing description, the composition may also contain other micronutrients of the type conventionally found in enteral compositions in accordance with EC Directive 1999/21/EC as well as flavourings such as coffee or vanilla, emulsifiers, thickeners and stabilizers of the type conventionally found in enteral compositions.

The nutritional composition may be produced by conventional methods. For example, the protein source and the lipid source are dissolved in water, preferably water which has been subjected to reverse osmosis, to form a liquid mixture. Emulsifiers may be dissolved in the lipid source prior to blending if desired. Preferably, a food grade emulsifier from a vegetable source is used.

The temperature of the water is conveniently about 50°C to about 80°C to aid dispersal of the ingredients. Commercially available liquefiers may be used to form the liquid mixture. Preferably, pH of the liquid mixture is adjusted to about 6.3 to 7 with food grade hydroxides.

After preparation of the liquid mixture, the carbohydrate source is added together with other easily dissolvable ingredients including, for example, vitamins, minerals, flavourings and colorants.

- 5 The liquid mixture may then be thermally treated to reduce bacterial loads (pasteurized). This may be carried out by steam injection or by heat exchanger; for example a plate heat exchanger.

- 10 If a shelf-stable liquid composition is required, an ultra heat treatment (UHT) is preferably conducted after pre-heating to 50-85°C. For example, an indirect UHT treatment may be conducted at 140-155°C for 5-8s, in a tube heat exchanger. The liquid mixture may then be cooled to about 60°C to about 85°C; for example by flash cooling. The liquid mixture is then homogenized and the resulting homogenised milky liquid may be aseptically filled into suitable containers such as 200 ml cups for
15 oral feeding. Aseptic filling of the containers may be carried out by cooling the liquid mixture.

- If a powdered, reconstitutable formula is required, the homogenised mixture can be evaporated and dried to powder; for example by spray drying. Conventional
20 procedures may be used.

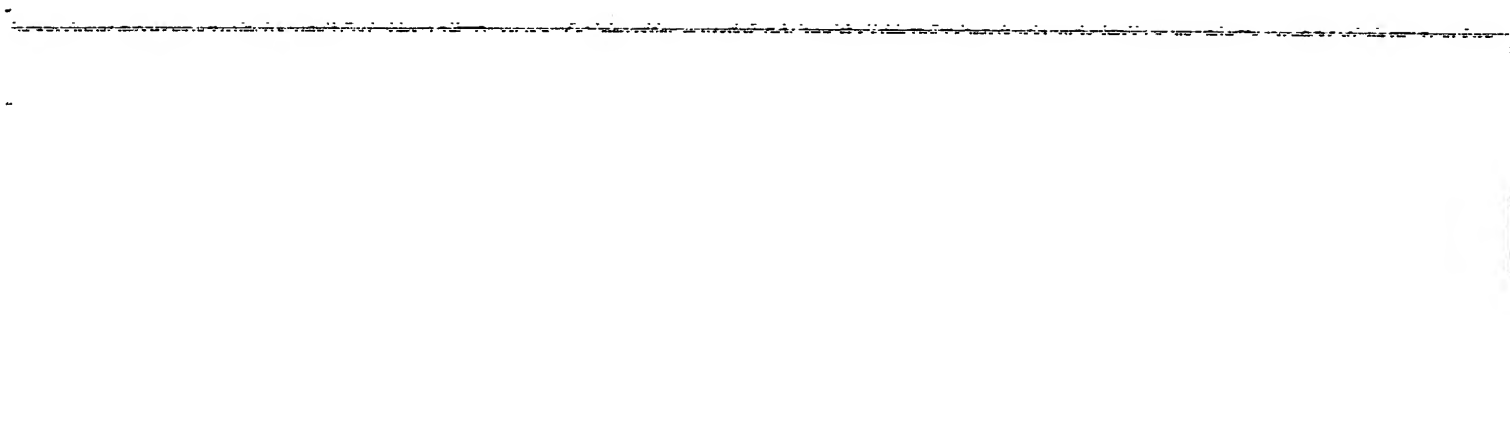
Experimental Example

- 25 Normal human fibroblasts were trypsinised and seeded in 12 well plates at a density of 10,000 cells/cm³. When confluent, the cells were transferred to a culture medium with an amino acid distribution and concentrations designed to mimic those in human serum as closely as possible. The cell cultures were divided into two categories, a control culture in which the culture medium contained 0.201 mM proline and an
30 experimental sample in which the culture medium contained 0.592 mM proline. After 24 hours fibroblast-conditioned medium containing 100 microgram/ml beta-aminopropionitrile to prevent cross-linking of collagen molecules in the cultures was collected. The conditioned medium was dotblotted to a nitrocellulose membrane and probed for collagen type I content with a polyclonal immune-absorbed antibody. The

value shown for the proline-supplemented samples is relative to the controls set at 100%.

5	Sample	% of control value
	Control (0.201 mM Proline)	100%
	Proline-supplemented (0.502 mM Proline)	150% \pm 21.9%

10 This experimental example shows that human fibroblasts respond to proline supplementation with a 50% increase in collagen synthesis. In this proline-supplemented medium, increased collagen synthesis is independent of the addition of growth factors or other mediators stimulating collagen transcription. It indicates an increased substrate requirement for efficient collagen synthesis.



Claims

1. A nutritional composition for promoting wound healing comprising a protein source, a lipid source and a carbohydrate source wherein no more than 1.8% of the total calories of the composition derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the composition..
2. A nutritional composition according to Claim 1 wherein at least 3.5% of the total calories of the composition derive from proline.
3. A nutritional composition according to claim 1 or 2 wherein 1.5% of the total calories of the composition derive from arginine.
4. A nutritional composition according to any preceding claim wherein the protein source constitutes at least 28% of the total calories of the composition.
5. A nutritional composition according to any preceding claim which composition has an energy density of about 1.25kcal/ml.
6. A method of providing nutritional support to a patient with an acute or chronic wound comprising the step of administering a therapeutically effective amount of a nutritional composition comprising a protein source, a lipid source and a carbohydrate source wherein no more than 1.8% of the total calories of the composition derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the composition.
7. A method according to claim 6 wherein at least 3.5% of the total calories of the composition derive from proline.
8. A method according to claim 6 or 7 wherein 1.5% of the total calories of the composition derive from arginine.
9. The use of a protein source a lipid source and a carbohydrate source for the manufacture of a therapeutic formulation for promoting wound healing.

wherein no more than 1.8% of the total calories of the composition derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the formulation.

- 5 10. The use according to claim 9 wherein at least 3.5% of the total calories of the formulation derive from proline.
11. The use according to claim 9 or 10 wherein 1.5% of the total calories of the formulation derive from arginine

Abstract

5 A nutritional composition for promoting wound healing comprises a protein source, a lipid source and a carbohydrate source wherein no more than 1.8% of the total calories of the composition derive from arginine and wherein the protein source includes proline in an amount of at least 3% of the total calories of the composition. The composition may be administered orally and is particularly suitable for the amelioration of pressure ulcers although it may also be used with advantage in the nutritional management of acute wounds including pre and post surgery.



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